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Review

Current Progresses of Exosomes as Cancer Diagnostic and Prognostic Biomarkers

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Abstract

Cancer related exosomes are nano-size membrane vesicles that play important roles in tumor microenvironment. Emerging evidence indicates that exosomes can load unique cargoes, including proteins and nucleic acids that reflect the condition of tumor. Therefore, exosomes are being used as diagnostic and prognostic biomarkers for various cancers. In this review, we describe the current progresses of cancer related exosomes, including their biogenesis, molecular contents, biological functions, sources where they are derived from, and methods for their detection. We will also discuss the current exosomal biomarkers and the utilization of them for early diagnosis and prognostics in cancer.

Key words: exosomes, cancer, biomarkers, therapy, diagnosis, prognosis

Introduction

Cancer has been the major cause of death in the world. The survival rate is poor because of lacking early diagnosis and personalized treatment. Indeed, detection of tumors at early stages has been recognizing as a vital component of cancer-control for many years. Any strategies to achieve it will depend on the correct cancer detection markers, which exist in patients' tumor tissue and serum at various forms such as DNA, miRNA and proteins [1].

Exosomes, which were first identified by Johnstone in 1987 [2], are composed of a lipid bilayer, like cystic structure, ranging from 40 to 150 nm in diameter. Exosomes could carry materials into cells and cart stuff away, including nucleic acids, proteins and enzymes, suggesting useful, even life preserving functions. Consequently, tumor molecular and genetic cargoes, at least in part, are abundant in the body fluids or other associated tissues which could be used as cancer diagnostic and prognostic biomarkers. In this review, we highlight and discuss current progresses on cancer related exosomes and the application of exosomal markers for cancer diagnosis and prognosis.

Biogenesis and molecular content of exosomes in cancer

The biogenesis of exosomes starts when the cell membrane internalizes to form an early endosome. During this process, cytoplasmic content is constantly banding with inward budding of endosomal membranes to form exosomes. The endosomal sorting complex required for transport (ESCRT) machinery is required during this process. Members of the ESCRT family, tumor susceptibility gene 101 (TSG101) and ALG-2-interacting Protein X (Alix) proteins involve in it and are regarded as the core components of exosome lately in the literature [3]. Besides, exosomes can also form in ESCRT independent way which contains flotillin-2, CD63, CD81, ceramide and cholesterol molecules. After then, the endosome which is named multi-vesicular body (MVB), fuses with cellular membrane and releases exosome. The Rab family of small GTPase proteins, especially Rab27a and Rab27b, controls this step [4, 5]. But mechanism that drives exosome formation and secretion is largely unknown because of different cell types and their status.

Exosomes carry various molecular contents

which vary depend on the origin and their status [6] (Figure 1). For example, exosomes contains the characteristic proteins which belong to its biogenesis process, including TSG101, Alix, CD63, CD81 and Rab family proteins. All these molecules are often used as markers to confirm this vesicle. Exosomes also carry abundantly mRNA, DNA, microRNA(miRNA), long noncoding RNA (LncRNA) and other nucleic acid species inside. Besides, lipids are also an important part of the exosomes, such as cholesterol, phospholipids, glycerophospholipids and sphingolipids. They form the bilayer membrane structure and maintain its stable state [7]. Notably, in tumors, the molecular signature of tumor cells is enriched in exosomes that transfer between tumor cells and normal cells. Meanwhile, tumor cells may release more exosomes into microenvironment than normal cells, thus the circulating exosomes level is abundant.

Functions of cancer related exosomes

Exosome is a multifaceted regulator of cancer development. In multiple cancers, they usually harbor molecules derived from cancer, and are capable of changing the tumor microenvironment to effect neighbor cells or cells at specific distant sites. Therefore, they would be used as a tool to bridge normal cells to cancer cells and may facilitate the diagnosis of cancer [8]. To image the process of cell-to-cell communication, Suetsugu et al (2013) established cell and nude mouse models using green fluorescent protein (GFP)-tagged CD63. Labeled exosomes transferred from cancer cells to other cells in culture system. *In vivo*, GFP-exosomes are secreted into the tumor-surrounding tissue and circulate from primary tumors to metastatic niche [9].

First of all, exosomes play key roles in tumor growth and metastasis. Growth promoting gene could transported by exosomes and promote the proliferation of metastatic cancer cells. For example, epidermal growth factor receptor (EGFR)-containing exosomes favor this pattern to form liver-specific metastasis [10]. Scientists also found that exosomal miRNAs are capable of mediating silencing of downstream genes, which stimulate tumorigeneses in nontumorigenic epithelial cells [11]. Next, exosomes induce neoplastic proliferation or metastasis by evading cancer suppressors. Zhang L et al (2015) showed exosomes mediate tumor-suppressor phosphatase and tensin homolog (PTEN) transformation leads to PTEN loss in brain and enhance the outgrowth of brain metastasis [12]. Similarly, Putz et al (2012) reported PTEN protein could be directly brought out of cells and transferred to recipient cells as well as restore their function there [13]. It is also shown that exosomes are able to suppress glucose uptake by non-tumor cells to satisfy their own needs via secreting miRNA-122 high expressed-exosomes [14]. For another thing, exosomes are able to stimulate angiogenic activities [15]. In the study of Nazarenko et al (2010), when endothelial cells uptake tumor cells shuttled exosomal tetraspanin Tspan8, an important angiogenesis moderator, several angiogenesis-related genes of vascular endothelial growth factor (VEGF)-independent pathway would increase along with endothelial cell proliferation and migration [16]. Elucidation of the exosome uptake mechanisms is essential to understand the process of metastasis. A recent study demonstrated that exosomes derived from different tumors have their own "bias". The theory is that exosomes from lung, liver and brain-tropic tumor cells are uptaken by resident cells at the pre-metastasis niche due to their specific integrin [17].

Second, exosomes generated by cancer cells can stimulate immune response. However, they have a dual function in this aspect. On the one hand, exosomes from nasopharyngeal carcinoma can facilitate Treg recruitment, increase their suppressive function, and induce CD4 T-cells conversion, leading to immune system evasion from host immune surveillance [18]. Using fluorescently labeled exosomes, Wen et al (2016) found that exosomes suppress immune response via directly inhibiting T-cell growth and decreasing

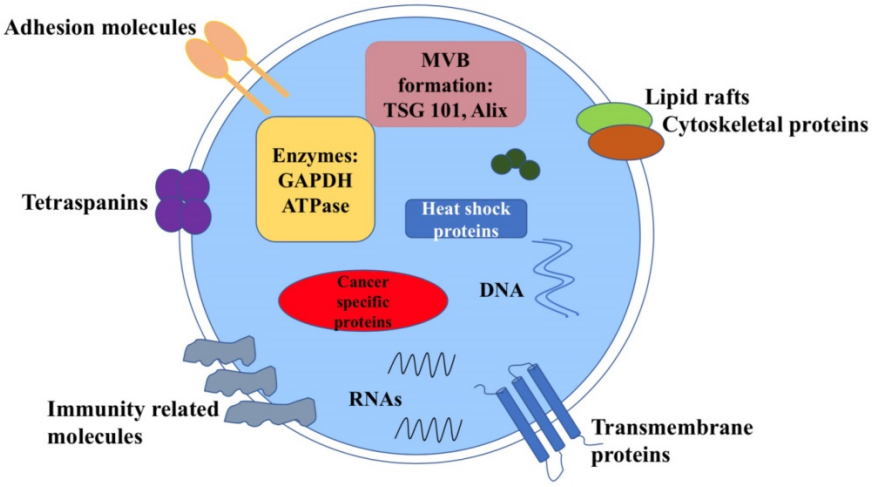

Figure 1. Structure and contents of cancer derived exosomes. Exosomes contain lipid bilayer membrane structures, which carry typical transmembrane proteins and receptors, adhesion molecules, the lipid raft associated protein, immune regulator molecules and tetraspanins. Within the exosomal lumen, several proteins and nucleic acid can be found, including a vast array of different molecules depending on different types of cancer cells.

Natural killer (NK) cell cytotoxicity [19]. Further, Mizyazaki et al (2018) reported that overexpressing estrogen receptor-binding fragment-associated antigen 9 (EBAG9) in exosomes and transferring them to T-cells can activate immune escape and cell migration more potently [20]. On the other hand, exposing tumor cells to stress may improve the antitumor immunity. After being heat stressed, exosomes from carcinoembryonic antigen (CEA)-positive tumor cells have higher immunogenicity, suggesting they could be used as a new effective vaccine for cancer immunotherapy [21].

In recent years, mounting evidence has suggested that exosomes play an important role of mediating resistance to therapy. Indeed, exosomes can transfer resistant phenotype from drug resistant cells to drug sensitive cells, through transferring of drug-efflux pumps and inclusive RNAs [22]. To detect how exosomal miRNAs contribute to the development of drug resistance, Fabbri et al (2015) designed a co-culture system using neuroblastoma cells and human monocytes. They found that miR-155 raised about four folds in this tumor cells since the exosomal transformation of monocytes, accompanied by increased cisplatin resistance via depressing its target shelterin component TERF1 [23]. Similarly, Qu et al (2016) showed that exosomes could also confer the sunitinib resistant phenotype to renal cancer cells. Activated exosomal LncARSR has been proved as the inducer of this process [24].

This cross-talk is also reflected between cancer stem cells and the normal cells, including fibroblast, endothelial cell and mesenchymal cell. Cancer stem cells (CSCs) are considered as cornerstone, which can contribute to tumor progression, therapy resistance and metastasis - and their associated exosomes - have been implicated in various cancer. It is now thought that exosomes may up-regulated mRNA, protein expression of related genes in recipient cells [25-27]. For example, pluripotent stem cell-derived mesenchymal stem cells derived exosomes enhance the proliferation, migration capacities of endothelial cells by activating phosphatidylinositol 3-kinases (PI3K)/AKT pathway to exert a preventative effect [28]. As the research moves along, more puzzles will be unraveled. Some functions of exosomes in cancer are summarized in Figure 2.

Sources of cancer related exosomes

Body fluids derived exosomes

Oncologists first separated cancer cell derived exosomes from mice and patient's blood, which are most common sources for exosomes and reflect the function as pro- or anti-tumor effectors, including immune response, tumorigenesis, cell migration and invasion [29]. Platelet-derived extracellular vesicles (P-EVs) is the most abundant specie in human blood, accounting for two-thirds of peripheral blood extracellular vesicles [30]. Many studies highlight that P-EVs participate in several intracellular processes, including homeostasis, thrombotic and cancer progression [31, 32]. Based on the study of Liang et al (2015), miR-223 in the platelet-secreted extracellular vesicles from patients with hematogenous metastatic lung cancer is more abundant than healthy subjects [33]. Janowska et al (2005) also showed that P-EVs are capable to promote proliferation and invasion in lung carcinoma cell lines by activing mitogen activated protein kinase (MAPK) pathway or stimulating matrix metalloproteinases (MMPs) [34]. In addition, in response to the stress of inflammation, more P-EVs could be released [35]. Altogether, P-EVs might be useful for the development of novel cancer biomarkers and therapy strategies in the near future.

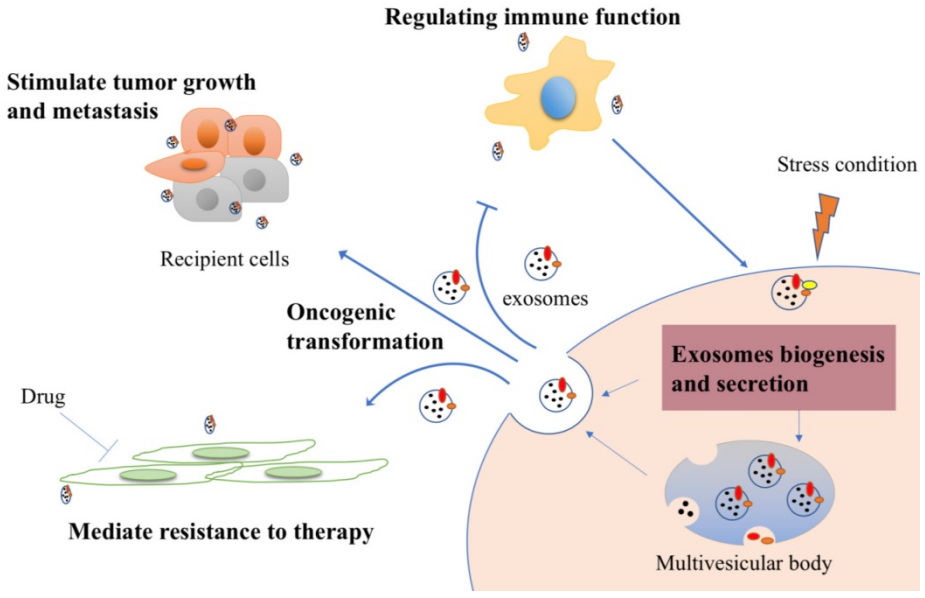

Figure 2. Cancer derived exosomes-mediated cellular process signaling. Tumor exosomes carry proteins and nucleic acids and are released into tumor microenvironment. They could be uptaken by recipient cells to stimulate tumor growth and metastasis. After drug treatment, exosomes, if they derived from drug resistance cells, can also transmit the resistant feature to recipient cells to mediate resistance. Furthermore, exosomes can stimulate immune response to inhibit immune cells response in general, while increase immunogenicity to improve anti-tumor effect under some specific stress conditions.

In the field of cancer research, however, two other sources of exosome have been discovered. For the urine exosomes, researchers demonstrated that exosomes could be detected from normal volunteers or patients with kidney disease [36]. For instance, after proteomic analyzing, 295 proteins were revealed, including multiple protein products of genes already known to be responsible for renal and systemic diseases, providing a potential tool to discovery of cancer in early stage [37]. In prostate cancer, McKiernan et al (2016) compared the efficiency of combining urine exosome gene expression assay with standard of care (SOC) or SOC alone. This novel assay plus SOC improve the accuracy and sensitivity of prostate cancer screening and diagnosis [38]. Beyond this, it is conceivable that urine exosomes could be used for low-cost screening for early detection of cancer and therapeutic monitoring. Nevertheless, because of glomerular filtration, only unique size (<100 nm) of exosomes could be detected [39, 40].

For the breast cancer patients, breast milk exosomes uniquely contribute to cancer detection. Qin et al (2016) detected the expression of six proteins linked to breast cancer in three milk samples (transitional, mature, and wean). The results showed that transforming growth factor β2 (TGFβ2) level increased in both wean and early involution, suggesting high TGFβ2-expressing breast milk exosomes have higher cancer risk [41]. Yu et al (2017) discovered the expression of miR-29b and miR-21 are high in milk-derived exosomes, which may activate the MAPK signaling and promote cell growth [42]. Besides, it was shown that human breast milk exosomes are connected with immune system. For example, exosomes, which are isolated from human colostrum and mature breast milk, may inhibit inflammatory factor production from allogeneic and autologous peripheral blood mononuclear cells as well as increase the number of microRNAs to participate in the process of immune regulation [43, 44]. Consistent with a prior research, about 67.82% well-characterized immune-related pre-miRNAs are detected in breast milk exosomes which could be transfer from mother's milk to the infant to develop immune system [44]. Hence, scientists begin to design the drug-loaded exosomes to improve chemotherapeutic drug efficacy and reduce toxicity. However, the discipline of cancer related exosomes in human breast milk is still not clear.

Tissue derived exosomes

Currently, the studying of this source of exosomes is still in the early stage. Gallart et al (2016) have successfully enriched the extracellular vesicle from tissues of central nervous system. They presented that more than 86% exosomal markers could be identified in brain exosomes [45]. Vella et al (2017) also reported this kind of exosomes, which are extracted from human brain tissue, maintain the same traits of brain homogenate by synthesizing proteomic and genomic profiles [46]. It suggests that exosomes released from tissue might act as a new effective resource. In Teng et al (2017) study, high level of tumor suppressor miRNAs, such as miR-193a, miR-18a and miR-155, were selectively packaged by exosomes and expelled from advanced colon cancer cells, thereby resulting in high accumulation in circulation [47]. Similarly, for human renal tissue, comparing with normal renal, carcinoma tissues highly express azurocidin to promote angiogenesis, but it was not related to tumor stage or grade [48]. However, regardless this deficiency, the analysis of such exosome derived cargoes still reveals the novel mechanism of tumorigenesis and is starting to be useful to monitor tumour procession as well as drug response. Furthermore, there are two further advantages. First, since they are directly isolated from tissue which effected by the complex tumor microenvironment, exosomes more accurately reflect the status of tumor or human body. For instance, although exosomes maintain cellular homeostasis by activating cytoplasmic DNA sensing response *in vitro*, liver tissue derived exosomes contribute more markedly *in vivo* [49]. Second, body fluid samples contain more contaminants, including serum abundant proteins, metabolite and even other derived exosomes. In contrast, they come directly from the origin and do not need to be transferred, thus allowing cancer cell exosomes avoid degradation and contamination. Meanwhile, the difference of post-mortem and storage time as well as the number of freeze-thaw cycles may challenge the quality and efficiency [46]. Nowadays, Mincheva and colleagues have established the protocol to isolate exosomes from tissue explants in 2016. They cut fresh tissue into small pieces and culture them for a short time. By centrifuging the collected culture medium, exosomes will be enriched [50]. But due to the different property of tissues, different culture medium or dispose method should be considered to maintain the integrity of exosomes and their cargoes.

Exosome based biomarkers in cancer

It appears that blood profiling from cancer patients has the value to capture tumor status. Indeed, since Gold demonstrated the role of glycoprotein antigen in detecting human colon cancer in 1965, lots of serum cancer biomarkers have been commonly used in practical clinical realities [51]. For example, carbohydrate antigen-and125 (CA-125) presents its

potential for monitoring response to ovarian cancer diagnosis about 20 years ago. But it has low sensitivity, which is less than 50% for early-stage ovarian cancer and more than 20% false negatives value, and it also reveals high false positive value when the patients with benign gynecological diseases are tested [52]. Besides, there is a degradation reaction whose speed was uncontrollable in all kinds of serum tumor markers because they completely exposed to the blood. Therefore, although it is a good point to use a combination of more biomarkers to upgrade the overall sensitivity and specificity, the benefit is still far more away. Currently, to address this problem, circulating tumor cells (CTCs) have attracted interest. It carries the molecular and phenotypic information of tumors and can be easily obtained from blood. Nevertheless, they are extremely rare within an overwhelming background, which is 1 mL of whole blood contains about 5 million mononuclear cells, and only 1.43% of progressive breast cancer patients had more than 500 CTCs in 7.5mL blood [53-56]. Moreover, many issues regarding the mechanism of immune system or lymphatic spread remain unknown. But they are the crucial route for CTCs transfer, degradation, and even elimination [57]. In contrast, exosome contains the molecules that strongly reflect the parental property, and has the advantages that they are very abundant in blood, highly stable and could be analyzed in small volumes (100 µL) of frozen serum or plasma samples, which could be used as a new source of new biomarkers for personalized diagnosis and prognosis [58].

Exosomes carry multiple protein cargos, which have been explored from studies of human pancreatic cancer, lung cancer, colorectal cancer and melanoma (Table 1). For pancreatic cancer, Melo et al (2015) found glypican-1 GPC1+ exosomes show a higher proportion in pancreatic cancer patients (100%) than healthy people (average of 2.3%). Notably, they also found this marker revealed perfect sensitivity and specificity in early stage of pancreatic cancer [59]. Another signature in exosomes, macrophage migration inhibitory factor (MIF), was highly expressed in stage I patients with high liver metastatic potential [60]. In the case of treatment response of locally advanced pancreatic cancer, An et al. demonstrated that exosomal vimentin level was decreased after gemcitabine treatment, which means it can be used as a candidate for cancer prognosis [61]. Similarly to pancreatic cancer, EGFR is higher enriched in exosome from gastric cancer patients, thereby, making it a potential marker [10]. However, the abundance of exosomal specific proteins are very low, large volume serum or culture medium is required to isolate enough exosome to do the further studies neither proteomics or identification via western blot. Thus, exosome protein profile is still in infancy.

Genomic profile of the exosome, including miRNA, LncRNA, messenger RNA and mitochondrial RNA, appears to be a potential biomarker source to classify tumor types (Table 2). They can be amplified by PCR which makes up for the shortcoming of quantity, and therefore makes them as an excellent tool for cancer diagnose. For instance, Fang et al (2018) showed hepatoma cells exhibited high level of miR-103 and could deliver it into endothelial cells though exosomes to induce metastasis, suggesting secreted miR-103 has the capacity to be a predictive marker [62]. Shi et al reported exosomes-delivered miR-638 was obviously down-regulated in serum of hepatocellular cancer patients, with larger tumor size (> 5 cm) or at later TNM stage (III/IV) [63]. Furthermore, long noncoding RNAs, like lnc-sox2ot, lnc-h19 and LncRNA-ARSR, have been investigate in circulation exosomes and highly correlated with tumor stage and overall survival rate of patients [24, 64-68]. These findings demonstrate that it is possible to find exosomal biomarkers from circulation or other sources to evaluate cancer invasion and progression, with higher sensitivity and specificity.

Methods available for biomarker detection in cancer exosomes

To enhance specificity and availability of exosomal biomarkers, a variety of methods are developed. Over the past decade, mass-based proteomic analysis is used to identify protein species in exsomes. For instance, higher 27-Hydroxycholesterol level in estrogen receptor positive (ER+) breast cancer cell line (MCF-7) exosomes has been detected by liquid chromatography-mass spectrometry [69]. Furthermore, Hyun et al (2013) combined microscopy and mass spectrometry to map endogenous proteins in living cells. They successfully identified 495 proteins in mitochondrial matrix after labeling the peroxidase enzyme nearby the target proteins. This study outlines a new approach that may be valuable in the field of exosomes. However, because of the contaminant proteins, there are still some challenges to truly define the exosome proteomic map [70]. Researchers also developed tandem-mass-tag (TMT) quantitative proteomics approach and support vector machine cluster analysis. This approach highlights the protein abundance rather than protein presence. They thought the approach should be useful for identifying and confirming new protein candidates in exosome [71].

Table 1. Exosomal protein markers in multiple cancers

Tumor types	Body fluids	Enrichment method	Signature or pool in exosomes	Application	Ref.
Pancreatic cancer	Patients' blood	Ultracentrifugation	Glypican-1	Diagnosis	[59]
	Medium	Ultracentrifugation	CD151, ADAM1 and ADAM15	Prognosis	[91]
	Patients' plasma	Ultracentrifugation	MIF	Prognosis and treatment	[60]
	Patients' serum	Ultracentrifugation	vimentin	Diagnosis and treatment	[61]
Melanoma	Patients' plasma	Ultracentrifugation	TYRP2, VLA-4, HSP70, HSP90 and MET	Prognosis and treatment	[92]
	Patients' serum	Ultracentrifugation	MIA and S100B	Prognosis and diagnosis	[93]
Lung cancer	Urine	Ultracentrifugation	LRG1	Diagnosis	[94]
	Saliva	Ultracentrifugation	BPIFA1, CRNN, MUC5B, and IQGAP	Detection	[95]
	Medium	ExoQuick-TC™ kit	Vasorin	Treatment	[96]
	Patients' blood	Ultracentrifugation	LG3BP and PIGR	Diagnosis	[97]
Glioblastoma	Medium	Ultracentrifugation	angiogenin, IL-6 and IL-8	Detection and diagnosis	[98]
Gastric cancer	Medium	Ultracentrifugation	EGFR	Detection and diagnosis	[10]
Colorectal cancer	Patients' blood	Ultracentrifugation	CD147	Detection and diagnosis	[99]
	Patients' plasma	ExoCap™ kit	GPC1	Diagnosis and treatment	[100]
Ovarian cancer	Ascytic fluid	Ultracentrifugation	CD24	Diagnosis	[101]
	Ascitic Fluid	Ultracentrifugation	TGM2, U2AF1, U2AF2, and HNRHPU	Diagnosis	[102]
Cholangiocarcinoma	Patients' blood	Ultracentrifugation	VNN1, CRP, FIBG, IGHA1, and A1AG1	Diagnosis	[97]
Prostate cancer	Urine	Ultracentrifugation	PCA3	Diagnosis and monitoring	[103]
Glioma	Cerebrospinal fluid	Ultracentrifugation	IL13QD	Detection and recurrence	[104]

Table 2. Exosomal nucleic acids markers in multiple cancers

Tumor types	Source	Enrichment method	Signature or pool in exosomes	Application	Ref.
Pancreatic cancer	Patients serum	Ultracentrifugation	miR-1246, miR-4644, miR-3976 and miR-4306	Diagnosis	[105]
	Postoperative blood		LncRNA-Sox2ot	Prognosis	[64]
	Patient plasma	Ultracentrifugation	miR-10b	Detection, prognosis	[106]
	Patients' blood	Ultracentrifugation	miR-10b, miR-21, miR-30c, miR-181a, miR-let7a	Detection	[107]
	Patients urine	Ultracentrifugation	PCA-3 mRNA	Diagnosis and monitoring	[103]
	Patients serum	Ultracentrifugation	KRAS DNA	Detection, prognosis	[108]
Hepatocellular carcinoma	Serum	ExoQuick Kit	miR-18a, miR-221, miR-222, miR-224	Diagnosis	[109]
	Patients serum		miR-638	Detection, prognosis and recurrence	[63]
	Patients serum	Ultracentrifugation	miR-718	Detection, prognosis and recurrence	[110]
	Medium	ExoQuick Kit	miR-122	Potential treatment	[111]
	Medium and mice blood	Ultracentrifugation	miR-103	Potential treatment	[62]
	Mice serum	Ultracentrifugation	CDYL circRNA	Diagnosis	[112]
	Medium	Ultracentrifugation	LncRNA-H19	Treatment	[65]
Renal cell carcinoma	Medium	Ultracentrifugation	LncRNA-ARSR	Potential treatment	[24]
Glioblastoma	Patients serum	Ultracentrifugation	EGFRvIII (mRNA)	Diagnosis and treatment	[98]
	Patients serum		LncRNA-HOTAIR	Prognosis and diagnosis	[66]
Melanoma	Medium and mice plasma	Ultracentrifugation	BRAF(DNA)	Detection and treatment	[113]
Lung cancer	Medium and mice plasma	Ultracentrifugation	EGFR(DNA)	Detection and treatment	[113]
	Patients serum	Ultracentrifugation	miR-1247-3p	Diagnosis	[114]
Colorectal cancer	Patients serum	Ultracentrifugation	circ-KLDHC10	Diagnosis	[112]
	Patients serum	Ultracentrifugation	BRAF and KRAS(mRNA)	Detection	[115]
	Mice serum	Ultracentrifugation	miR-193a	Detection	[47]
	Patients serum		LncUEGC1 and LncUEGC2	Diagnosis	[67]
	Patients serum	Ultracentrifugation	miR-19a	Prognosis and recurrence	[116]
Prostate cancer	Patients plasma	ExoQuick Kit	miR-1290 and miR-375	Prognosis	[117]
	Serum and urine	ExoMiR extraction kit	miR-141 and miR-375; miR-107 and miR-574-3p	Diagnosis	[118]
	Urine	EXOPRO Urine Clinical Sample Concentrator Kit	ERG, PCA3, and SPDEF (RNA)	Diagnosis	[38]
	Urine	Ultracentrifugation	miR-196a-5p and miR-501-3p	Diagnosis	[119]
Ovarian cancer	Patients serum	modified magnetic activated cell sorting (MACS) procedure	miR-21, miR-141, miR-200a, miR-200c, miR-200b, miR-203, miR-205 and miR-214	Diagnosis	[120]
	Blood	Exosome Isolation Reagent	*miR-21*	Diagnosis	[121]
	Urine	Ultracentrifugation	*miR-30a-5p*	Diagnosis and treatment	[122]
Bladder cancer	Urine		miR-720/3007a, miR-205, miR-200c-3p and miR-29b-3p	Detection	[123]
Gastric cancer	Patients serum		*LncRNA HOTTIP*	Diagnosis and prognosis	[68]

In addition, exosomal enriched gene information is another potential biomarker source. Present technology such as RNA-sequencing and DNA sequencing [72] already enables analysis of genomic information of exosomes. Work carried out by Michal et al (2013) identified third putative Dis3p homologue (hDIS3L2), which causes altered levels of multiple mRNAs, while it is not a part of exosomal RNA [73]. Matthew et al (2017) reported that mounts of small noncoding RNA species were mapped via sequencing technology, including transfer RNAs, long noncoding RNA and small nucleolar RNAs. Then, they also confirmed the tRNAs took part in exosome-specific posttranscriptional modification [74]. It is believed that next generation sequencing is able to define the global or specific RNA profile of serum exosomes [75]. Similarly, DNA sequencing offers the opportunity to accurately detect profiling of the genomic landscapes of cancer cell derived exosomes, make an excellent tool to diagnose and judge the prognosis. A proof-of concept study by Lucas et al (2016) demonstrated this method and presented 95-99% of target regions covered at a mean depth of 133-490x, including multiple actionable mutations, such as notch receptor homologs (NOTCH1) and BRCA2 in patient exosomal DNA [76]. To summarize, the time has come for a better understanding of the characterization and the function of the exosomal genomics, which is beneficial for cancer detection.

Clinically relevant exosomal biomarkers for breast cancer

This pattern has attracted enormous attention of researchers resulting in the discovery of exosomal signatures with diagnostic and prognostic relevance for many types of cancer. Because cancer is a heterogeneous disease, here we choose breast cancer as an example.

Exosomal proteins

Protein values of exosomes from cancer patients are higher than normal and they reflect the cancer stage, especially the proteins derived from cancer cells (Table 3). In the study of Moon et al (2016), fibronectin level is significantly high in plasma at early stage of breast cancer patients, with sensitivity of 65.1% and specificity of 83.2% [77]. Further, levels of nephronnectin, a key factor known to promote adhesion and anchorage-independent growth, was found to be overexpressed in tumor tissue exosomes of breast cancer patients, associating with poor outcome in patients with luminal A subtype [78]. Besides, Khan et al (2014) showed survivin and its variant were detected in serum exosomes, and significantly higher in early stage breast cancer patients [79]. Another marker, extracellular matrix metalloproteinase inducer (EMMPRIN) has been enriched in tumor-derived microvesicles, also present high correlation with metastatic patients [80]. Based on these data, screening the different proteins of all breast cancer cells types, or looking for the same one of different cancer cells would increase the sensibility and specificity to promote cancer early detection, diagnosis and intervention.

Nucleic acids in exosomes

Besides from its specific protein, exosomes also carry a select set of functional circulating nucleic acids such as mRNAs, miRNAs, LncRNAs and DNA [81]. Exosomes are the main source of circulating miRNAs. So far, a great number of studies have analyzed the major miRNAs that show the perspectives to be diagnostic biomarkers in breast cancer. The results of weiying Zhou et al. indicate that miR-105 highly expressed in circulation at the premetastatic stage or distant metastatic stage. They also showed that this molecule and miR-181a predominantly existed in serum exosomes of stage II and III breast cancer patients [82]. Furthermore, increased invasion and proliferation are positively correlated with overexpression of exosomal miR-21 and miR-1246 in breast cancer [83]. In addition, other researchers also demonstrated that exosomes contain much miRNAs, such as miR-101, miR-372 and miR-130a-3p, which could be transferred from tumor to recipient cells, resulting in tumor progression and metastasis and presenting opportunity incorporating them into clinical study [84, 85].

Another non-coding RNA, long noncoding RNAs (LncRNAs), has a more complex structure which could bind proteins, RNA and DNA, and play important roles in life process ether normal or disease [86]. The use of exosomal LncRNAs as a potential biomarker in breast cancer was first reported by Lu et al (2015). In this study, LncRNA RP11-445H22.4 is highly expressed in cancer tissues and serum from breast cancer patients compared to healthy controls, with a sensitivity of 92% and a specificity of 74%. Notably, they found that the level of circulating this molecule is more correlated with estrogen receptor and progesterone receptor than human epidermal growth factor receptor-2 state [87], indicating that LncRNA RP11-445H22.4 could be used as a novel complementary marker for breast cancer diagnosis and prognosis. The accumulating evidence for the unique nucleic acids signatures of exosomes is increasing (Table 4), but much work still awaits confirmation of their clinical responses in future studies.

Table 3. Clinically relevant exosomal protein markers for breast cancer

Proteins	Source	Detection method	Patient stages	Application	Diagnostic value/outcome	Ref.
Fibronectin	Plasma	ELISA	Early stage (0, I and II)	Diagnosis	Sensitivity of 67.7% and specificity of 72.0%	[77]
Nephronectin	Tissue	IHC microarray	Breast cancer (n=1393)	Prognosis	Sensitivity 70.8%	[78]
Survivin and Survivin-ΔEx3	Serum	Western blot	Stage (II - IV)	Diagnosis and treatment	NA	[79]
FAK	Plasma	Western blot	In situ and stages I-III	Diagnosis and prognosis	NA	[124]
EGFR	Plasma	Western blot	In situ and stages I	Diagnosis and prognosis	NA	[124]
EMMPRIN	Blood	Flow cytometry	Metastasis (n=8)	Prognosis	Median 31.7 % [IQR 21.35–37.35] vs median 19.5 % [IQR 10.03–24.88] in control	[80]
Periostin	Plasma	Western blot	Primary breast cancer with lymph node metastasis at diagnosis; relapsed metastasis during the follow-up	Diagnosis	NA	[125]
HER2	Plasma	Microfluidic chip	Breast cancer (n=19)	Diagnosis	NA	[126]
CD47	blood	Flow cytometry	Breast cancer (n=60)	Diagnosis	P=0.004	[127]
Del-1	Plasma	ELISA	Patients underwent curative surgery (n=115)	Diagnosis and treatment	94.8% patients showed a normalization of Del-1 lower than 0.5 after surgery and 10 patients showed Del-1> 0.4	[128]
TRPC5	Plasma	Flow cytometry	Unresectable metastasis (n=131)	Prognosis	P=0.0042	[129]
UCH-L1	Plasma	Flow cytometry	Resectable breast cancer (n=93)	Prognosis	P=0.009	[130]

Table 4. Clinically relevant exosomal nucleic acid markers for breast cancer

RNA/DNA	Source	Detection method	Patient stages and numbers	Application	Diagnostic value/outcome	Ref.
miR-105	Serum	RT-qPCR	Pre-metastatic and metastatic stage (n=75)	Detection and diagnosis	P = 0.04	[82]
miR-105 and miR-181a	Serum	RT-qPCR	Stage II, III (n=38)	Diagnosis	P < 0.01	[82]
miR-101, miR-372 and miR-373	serum	TaqMan MicroRNA assays	Invasive breast cancer (n=50)	Diagnosis	miR-373 -- higher in triple negative, estrogen-negative and progesterone-negative tumors	[84]
miR-1246 and miR-21	Plasma	RT-qPCR	Breast cancer (n=16)	Detection	The AUC for miR-21 was 0.69 (95 % CI 0.50, 0.88; p = 0.048) and for miR-1246 was 0.69 (95 % CI 0.49, 0.89; p = 0.068)	[131]
ssODNs	Plasma	ADAPT	Patients with positive breast cancer biopsy (n=59)	Diagnosis	P<0.05	[132]
miR-340-5p, miR-17-5p miR- 130a-3p, miR-93-5p	Plasma	PCR array	Recurrent (n=16)	Prognosis and recurrence	P<0.05	[85]
NANOG NEUROD1 HTR7 KISS1R HOXC	Plasma	PCR array	With or without first relapse and death (n=173)	Prognosis	P<0.05	[133]
LncRNA RP11-445H22.4	Serum	qRT-PCR	Breast cancer (n=68)	Prognosis	p < 0.001, sensitivity was 92% and specificity was 74 %	[87]

Conclusions and future perspectives

As mentioned above, targeting the exosomal cargos may express high diagnostic and prognostic potential. Indeed, numerous studies have attempted to explore the different profile and function of exosomes in health and disease, and to facilitate their clinical applications. But we are still on the early stage of exosomal research in cancer, especially breast cancer. Like other new biomarkers, cancer related exosomes also have disadvantages. The common method for exosome isolation is still ultracentrifugeation since it was first discovered. Although there are various alternative strategies that have emerged, including filtration, chromatography and bead isolation, the primary method remains purification by size [88]. This represents a disadvantage; some of protein fragments are enclosed in urine or serum during the isolated process, and therefore maybe directly disturb the results. Additionally, not only exosomes but also other extracellular vesicles such as microparticles and apoptotic bodies carry bioactive molecules [89, 90]. An outstanding question remains as to whether body fluids or tissue derived exosomes sufficient purenesses are needed as a result. Furthermore, for the exosomal content, the mechanism of specific markers enrichment is not fully understood. Most studies just analyzed one kind of exosomal contents at specific time point but neglected other factors. However, any ideal biomarkers must be

unique and sensitive for tracing upon different stages of cancer. Thus, it is essential that scientists continue exploring the secret of this field and establish of standard operating procedures related to exosome analysis for successfully improve veracity and enrich repeatability into clinically meaningful tests. With more and more novel exosomal markers continuing to emerge, it will spark up hope for realistic tracking of exosome release and uptake, even combining cancer early detection and exosome-based drug delivery to improve patients' survival rate. Thus, beyond these considerations, further quantitative data which focus on the metastasis niche and its function in tumor development, as well as more subtle biological structure of exosomes will achieve the clinical utilization of exosome as a specific biomarker. The future application of exosomes as cancer early detection and prognostic biomarkers will certainly be a new weapon to win this fight with cancer.

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Competing Interests

The authors have declared that no competing interest exists.

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